The effect of stimulus saliency on the modulation of pain-related ongoing neural oscillations: a Registered Report

Chiara Leu[1]*, Sébastien Forest[1], Valéry Legrain[1,2,3], Giulia Liberati[1,2]

[1] Institute of Neuroscience, Université catholique de Louvain, Brussels, Belgium

[2] Psychological Sciences Research Institute, Université catholique de Louvain, Louvain-la-Neuve, Belgium

[3] **Louvain Bionics, Université catholique de Louvain, Louvain-la-Neuve, Belgium**

*Corresponding author. Chiara Leu, Institute of Neuroscience (IONS), Université catholique de Louvain, 53 Avenue Mounier, 1200 Bruxelles, Belgium.

Email: chiara.leu@uclouvain.be

Abstract

Ongoing oscillations have been shown to be modulated in different frequency bands following phasic, tonic as well as periodic thermonociceptive stimulation. Yet, it remains unclear whether these modulations are related to pain perception, saliency (i.e., the ability of a stimulus to stand out from its environment) or solely the intensity of these stimuli. To better understand this relationship, we will combine a sustained periodic thermonociceptive stimulation paradigm including periodic oddball events with a frequency-tagging analysis approach. Oddballs will be delivered either at a higher intensity or lower intensity (~~control~~ "high" vs "low" condition) than baseline stimuli. This will allow us to disentangle effects of saliency and intensity and investigate its relationship with pain perception and the modulation of ongoing oscillations. Continuous ratings of pain perception will be collected during the stimulation to track participants' perception. We expect to see a modulation of the EEG amplitude at the frequency of the oddball in both conditions in the theta, alpha and beta frequency bands. If the modulation is mainly driven by the intensity of the stimulus, we expect that the oddball in the ~~control~~ low oddball condition will have a lower amplitude than the ~~normal~~ high oddball. Conversely, if the modulation is reflecting the saliency of a stimulus, we expect the modulation at the frequency of the oddball to be similar across conditions.

Keywords: EEG, ongoing oscillations, saliency, pain, nociception, frequency tagging, oddball

1. Introduction

Saliency can be defined as the feature of a stimulus that makes it stand out from its environment (Egeth & Yantis, 1997). Painful stimuli emerge from the activity of the nociceptive system which is made to respond to high-intensity and potentially damaging somatosensory stimuli. These stimuli ~~and~~ are therefore inherently salient and ~~to~~ facilitate the involuntarily capture of attention (Eccleston & Crombez, 1999). The effects of saliency on event-related brain potentials (ERPs) evoked by nociceptive stimuli have been broadly studied (Iannetti et al., 2008 ; Legrain et al., 2003 ; Legrain et al., 2009 ; Roa Romero et al., 2013) , and evidence emerged that, in the experimental procedures in which they are usually elicited, the modulation of the magnitude of those ERPs can be mostly driven by the saliency of the eliciting nociceptive stimulus rather than its intensity and its painfulness. This dissociation between the saliency of the nociceptive stimuli and their ~~of stimulus intensity~~ painfulness ~~and saliency~~ was demonstrated, among others, by studies showing that the relationship between pain and ERP magnitude can be disrupted when nociceptive stimuli are repeated: repeating the stimulation reduces ERP magnitude while pain perception remains constant (Iannetti et al., 2008). Moreover, novel nociceptive stimuli elicit ERPs of larger magnitude and distract more participants from their primary task than stimuli of the same intensity but presented more frequently (Legrain et al., 2009).

Lately, it has also been shown that painful stimuli not only elicit ERPs, but also modulate the synchrony of ongoing neural oscillations in different frequency bands (Gross et al., 2007 ; Mouraux et al., 2003 ; Ploner et al., 2006 ; Schulz et al., 2011). Yet, it remains unclear whether these pain-related modulations of neural oscillations reflect changes in pain perception, stimulus saliency or merely objective stimulus intensity. Recent investigations were able to show the effects of bottom-up modulation on the modulation of ongoing oscillations by applying thermonociceptive stimuli of different intensities (Hauck et al., 2015 ; Tiemann et al., 2015 ; Zhang et al., 2012). While these studies provided evidence that the intensity of a stimulus modulates oscillations in the theta, alpha, beta and gamma frequency band, it remains

ambiguous whether the observed effects are related to the saliency of the applied stimuli or solely their intensity.

Using a frequency-tagging approach (Regan, 1989), investigations from our lab showed modulations of ongoing oscillations at the frequency of stimulation within different frequency bands following slow sustained periodic thermonociceptive stimulation (Colon et al., 2017 ; Liberati et al., 2019 ; Mulders et al., 2020). More specifically, in an investigation assessing intracerebral EEG recordings, Liberati et al. (2019) found a preferential modulation of thermonociceptive stimuli over vibrotactile stimuli in the posterior insula, in the alpha and theta frequency band. Yet, whether these differences in modulation are related to the painfulness, intensity or purely the saliency of the applied stimuli remains unclear. Further clarifying this relationship would be an important step to deepen our understanding of the potential association between the modulation of ongoing oscillations and pain perception. More specifically, this could tell us whether the observed neural modulations could indeed be a sign of a preferential modulation of painful stimuli rather than a response related to contextual and unspecific features such as stimulus intensity and saliency. Thus, the clarification whether the modulation of ongoing oscillations is more closely related to stimulus saliency or intensity would help to understand whether the modulation of ongoing oscillations could potentially be used as a physiological marker of pain in humans.

To shed light on the potential role of ongoing oscillations in the perception of salient stimuli, we will adopt an oddball paradigm during periodic nociceptive stimulation. Continuously oscillating thermonociceptive stimuli will be applied at the same location at a certain frequency, but every fourth stimulus will be presented at a higher stimulus intensity (creating the oddball effect, since these stimuli will “stand out” from the other stimuli). This effect will allow us to deliberately make some stimuli more salient than others and thus observe the corresponding brain responses, which we hypothesize are not merely related to changes in stimulus intensity. Previous studies using periodic visual stimuli have shown that oddball sensory events embedded in a regular series of stimuli (e.g., human faces among neutral objects, words

among nonwords, etc.) elicited in the EEG spectrum, in addition to the baseline response, a response peak specifically at the frequency of occurrence of those oddball stimuli (e.g. (De Keyser et al., 2018 ; Lochy et al., 2016 ; Rossion et al., 2015), analyzed using a frequency-tagging approach. To this date, no study has extended this oddball approach to the perception of painful nociceptive stimuli.

The aim of this study is to investigate whether changes in stimulus saliency induce a corresponding modulation of ongoing oscillations, and whether these modulations relate more closely to the saliency or the intensity of the stimulus. As saliency and stimulus intensity are inherently tied to each other, this investigation does not aim to quantify the exact contribution of each factor. More so, the goal is to achieve a better understanding of how both these factors (and their interaction) can modulate ongoing oscillations. ~~The saliency of the applied stimulus will be manipulated by occasionally changing its intensity. More specifically~~To this aim, intensity will be varied using an oddball paradigm during which the stimulation intensity changes periodically between baseline and oddballs which will be delivered at a higher stimulation intensity (i.e., “high” oddball). Based on Rossion et al. (2015), we expect to be able to “tag” both the baseline and the oddball response at their respective frequency of stimulation. To disentangle the effect of saliency and intensity (which are inherently tied to each other since a more intense stimulus is often also more salient), we will employ a control condition, during which the oddball will be delivered at the same frequency as in the high oddball condition, but with a *lower* stimulation intensity (i.e., “low” oddball). Thus, the main characteristic of this oddball will be its saliency, since its low intensity will make it different (i.e. “standing out”) in comparison to the baseline stimuli. We hypothesize that the oddballs in both conditions will be perceived at a different intensity compared to the stimulation at baseline frequency. Further, we expect that the oddball ~~in both conditions~~ will lead to a peak at its stimulation frequency for the high oddball condition. While we also expect a modulation for the low oddball if saliency affects the EEG response, no periodic modulation of this oddball would indicate a predominant role of intensity in the modulation of ongoing oscillations~~.~~ If the amplitude of the neural

response in the low oddball condition is similar to the amplitude at the oddball frequency in the high oddball condition, it would suggest that the modulation of ongoing oscillations is mostly affected by change detection rather than intensity. Conversely, if a periodic modulation is found in both conditions, but smaller for the low compared to the high oddball, the results would suggest that the modulation of ongoing oscillations is more closely related to the intensity of the stimulation, but still has an underlying contribution of the saliency of the stimuli. ~~, we expect to see a smaller to non-existent modulation at the oddball frequency in the low oddball condition.~~

2. Methods

2.1. Participants

We aim to recruit a gender-balanced group of 35 healthy adults that are between 18 and 35 years old (Creac'H et al., 2015). Participants will be recruited via an established website and social media and will be compensated with 25 € for the duration of the experiment (2 visits, lasting around 1.5h for the EEG assessment and 1h for the perception assessment~~each~~). The number of participants is based on a power and effect size estimation using the software G*Power (Faul et al., 2007). A more detailed sample size rationale can be found in the Supplementary Materials. Previous EEG investigations of bottom-up modulations of ongoing oscillations have recruited between 7 (Zhang et al., 2012) and 20 participants (Hauck et al., 2015 ; Tiemann et al., 2015), while investigations using a visual oddball paradigm with a frequency-tagging approach recruited 12 participants (De Keyser et al., 2018 ; Rossion et al., 2015) . Other pain-related frequency-tagging investigations recruited between 8 and 15 participants (Colon et al., 2017 ; Mulders et al., 2020). The experiment will be split into two separate visits to the lab; one to record EEG data and one to record continuous ratings during the same thermonociceptive stimulation paradigm. The order of the visits will be counterbalanced across participants.

Exclusion criteria will include regular use of psychotropic medication, intake of pain killers such as paracetamol, nonsteroidal anti-inflammatory drugs (NAIDs) or acetylsalicylic acid within 12h before the experiment, as well as any neurological diseases, psychiatric disorders, or recent upper limb trauma. The local Research Ethics Committee approved all experimental procedures (Commission d'Ethique Hospitalo-Facultaire, Saint-Luc Hospital & UCLouvain, B403201316436). Participants will be informed about all procedures and will have to sign an informed consent form prior to data acquisition. All procedures will be carried out according to relevant guidelines and regulations.

2.2. Thermonociceptive stimulation

Thermonociceptive stimuli will be applied using a contact heat thermode (TCS II, QST.Lab, Strasbourg, France) using a square probe (°T11) applied on the dominant volar forearm of the participant. The probe consists of 5 zones of 2 micro-Peltier elements each (~181 mm^2 per zone). The maximal heating ramp of this thermode is 75°C/s. The stimuli will be applied in a sustained periodic manner at a frequency of 0.5 Hz and oscillate between baseline temperature (35°C, approximately skin temperature) to a target temperature determined by the staircase procedure in the beginning of the visit (see section 2.3). The stimulation will be delivered over a period of 80s and the full stimulation surface will be used for each stimulation. The inter-stimulus-interval will be self-paced by the experimenter (min. 10s) and the thermode will be displaced after each trial to avoid habituation or sensitization.

2.2.1. Oddball paradigm

To introduce an oddball paradigm, every 4^{th} stimulus (oddball frequency: 0.125 Hz) will be delivered using a higher stimulus intensity (i.e., individual target temperature + 3°C) to make the oddball stimulus stand out from its environment. An illustration of the stimulation pattern can be found in Figure 1. A similar oddball paradigm using visual stimuli has been shown to elicit responses which can be easily identified using a frequency-tagging approach (Rossion et al., 2015). We also conducted a pilot study to ensure that the oddball would indeed be perceived as different from the baseline stimulation (see Supplementary Materials).

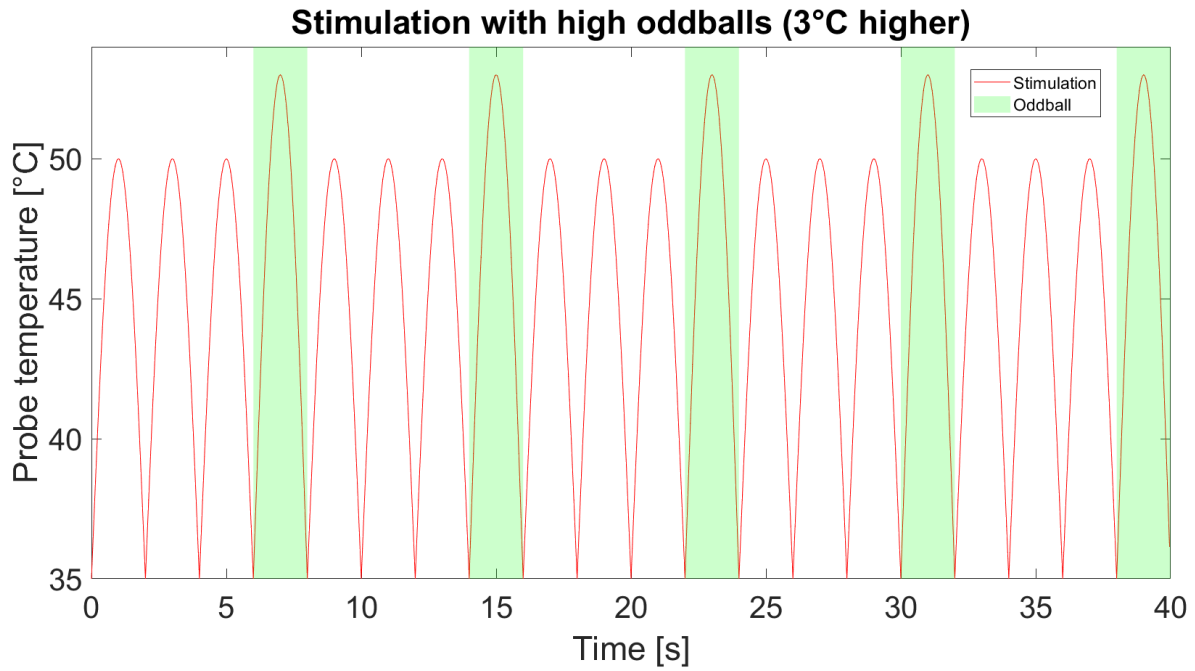


Figure 1: First half of the sustained periodic stimulation pattern during the "high oddball" condition with the example of 50°C as baseline target temperature. Every fourth stimulation (i.e., $F_{oddball}$=0.125Hz) will be delivered at 53°C, which is 3°C higher than the baseline stimulation. One trial consists out of 80s of stimulation (i.e., 10 oddball cycles).

2.2.2. Control condition

To disentangle whether possible effects (behavioral and in the EEG) are at least partially ~~actually~~ related to the saliency of the stimulus or rather to the change in stimulus intensity, a control condition (i.e., "low oddball") will be added to the experiment. To ensure that the oddball is still perceived as different but not more intense, the stimulation will be delivered at the same frequency as previously described (0.125 Hz), but at a *lower* intensity (individual target temperature - 3°C) than the baseline stimulation (Figure 2). For some participants, it is possible that this oddball will elicit a qualitatively different perception (i.e. not painful) compared to the other stimuli. While this could be considered a confounding factor, it is this attribute which will allow the stimulus to be salient, i.e. stand out from its environment (the painful baseline stimuli). We will then be able to compare behavioral and neural responses between the oddballs (high oddball vs low oddball), which are both salient (i.e., a change from the previous stimuli) but different in their intensity.

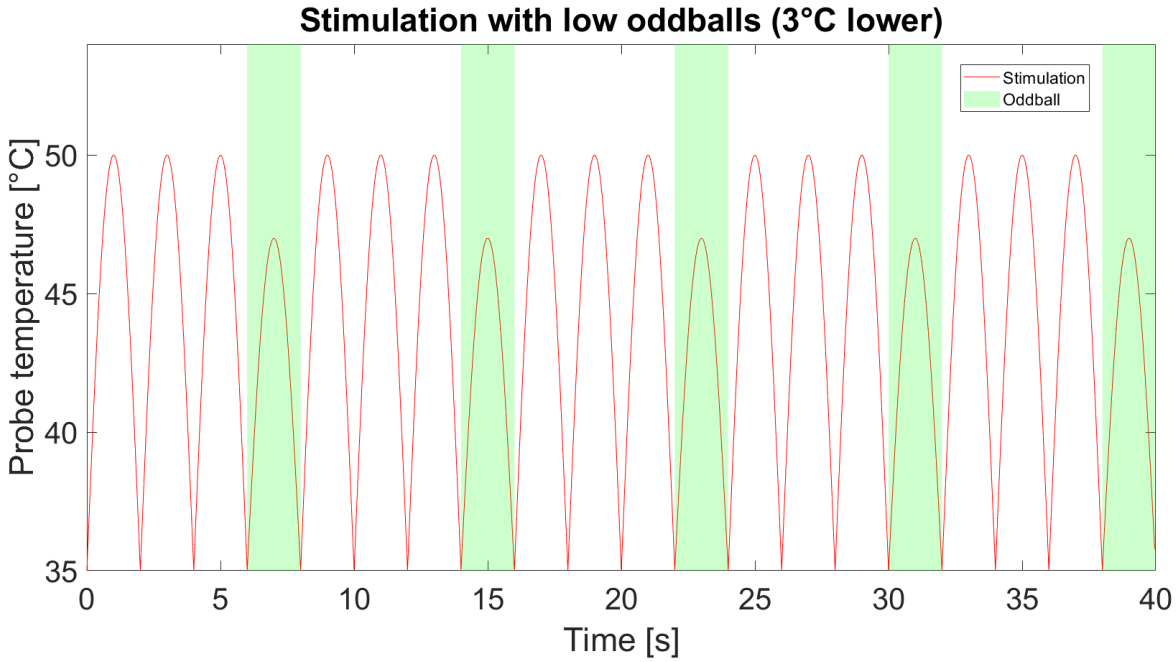


Figure 2: First half of the sustained periodic stimulation pattern during the "low oddball" condition with the example of 50°C as baseline target temperature. Every fourth stimulation (i.e., $FOS_{oddball}$=0.125Hz) will be delivered at 37°C, which is 3°C lower than the baseline stimulation. One trial consists ~~out~~ of 80s of stimulation (i.e., 10 oddball cycles).

2.3. Staircase procedure

A staircase procedure was implemented to identify the individual pain threshold to which the stimulation temperature of the baseline temperature will be adapted to. The aim will be to find a temperature which is tolerable for the full experiment (including high oddball trials), but still painful throughout the entirety of each trial (at the peaks of the stimulation). The stimuli applied in the staircase procedure were 40s long and were delivered the same periodic sinusoidal sustained fashion as the stimuli in the rest of the experiment, but without the addition of an oddball stimulus (illustrated in Figure 3). The first stimulus will always reach a temperature of 50 °C at every peak. Participants will be asked whether they perceived the stimulation as painful (at the peaks) throughout the 40s trial (if so, -0.5°C for the next stimulus), only painful in the first half of the trial (+0.5°C for the next stimulus) or as not painful ~~overall~~ (+1°C for the next stimulus). Participants will be instructed that painfulness relates to either a burning or pricking sensation (since we are predominantly stimulating C-fibers (Colon et al., 2017).The threshold for sufficient painfulness of the stimulation will be identified when a single step in temperature will lead to a change in perception of the painfulness in two consecutive trials~~twice~~

~~in a row~~ For example, the temperature is increased and the following trial is perceived as "generally painful". The next trial will have a 0.5°C lower stimulation temperature. If this lower trial is then perceived as "painful only in first half", we will choose the stimulation temperature of the preceding trial for the experiment. This temperature will then be used as the "baseline peak temperature", on which the stimulation temperatures for the high and low oddball depend on. The goal of this staircase is to reach a baseline stimulation temperature that will be perceived as VAS 5 or higher at its peaks during the entire 80s of the stimulation.

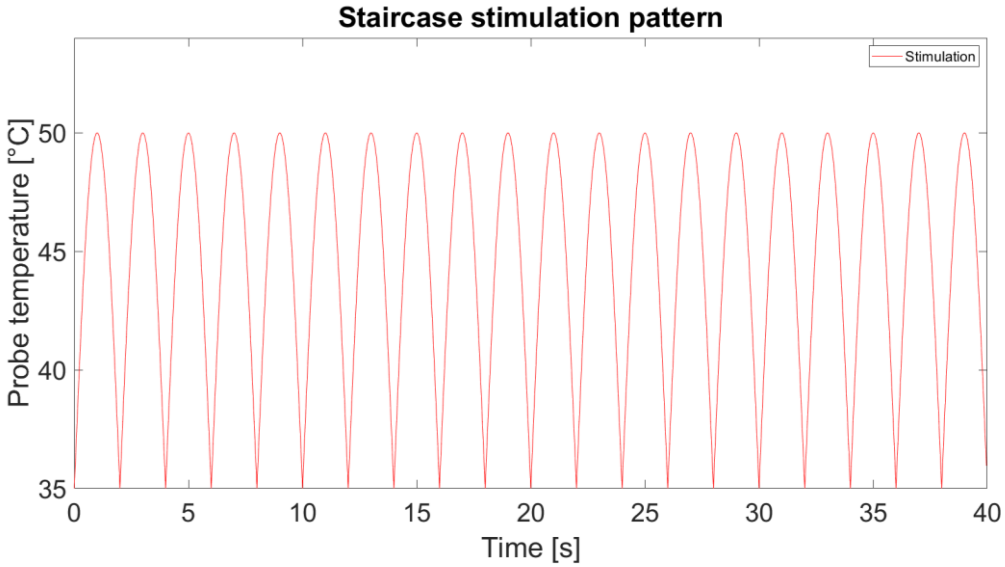


Figure 3: Illustration of the stimulation pattern used for the staircase procedure to identify the temperature at which the entire 40s trial is perceived as painful. The first trial will always be delivered with peaks at 50°C. The peaks of the following trials will depend on whether participants perceived the trial as "~~overall~~ generally ~~as~~ painful", "painful only in the first half" or "~~overall~~ generally ~~as~~ not painful".

2.4. Behavioral measures

Ratings of perceived stimulus intensity will be collected using a Visual Analog Scale (VAS) in the form of a slider implemented in a potentiometer. The continuous ratings will be digitized at 100 Hz with an analog/digital converter (USB-6343, National Instruments, Texas). No ratings will be collected during EEG data acquisition, as the arm movement would likely artifact the recordings. Thus, ratings will be collected in a separate visit during which no EEG data is acquired. Before the start of the VAS part of the experiment, participants undergo a familiarization phase during which warm innocuous stimuli will be delivered at the baseline stimulation frequency of 0.5 Hz onto the dominant volar forearm of the participant, while they

have to rate their perception on the VAS scale. This phase will not be considered for the analysis. The minimum of the VAS will represent “no perception” and the maximum will represent the “maximal pain imaginable”, while the middle of the scale will represent the threshold to pain perception. Participants will be asked to trace their perception using the VAS during each thermonociceptive stimulus following the familiarization phase. A pilot study examining whether participants would be able to trace the sustained periodic stimulation and detect the oddball stimuli in both conditions was conducted, a detailed description thereof can be found in the Supplementary Materials.

2.5. Experimental procedure

Participants will be seated comfortably in a chair during the experiment, while their dominant arm will be resting on the table with its volar surface upwards. They will be instructed to move as little as possible and keep their gaze constant to avoid interference with the EEG signal acquisition during the thermonociceptive stimulation. They will not be informed about the stimulation paradigm or any other details regarding the stimuli or the aim of the investigation. For each condition (i.e., ~~normal~~ high / ~~control~~low), 12 trials will be delivered distributed over 6 blocks of 4 thermonociceptive stimulation trials (Figure 4~~3~~). The breaks between the stimulation blocks will be self-paced, with a minimum of 2 minutes and a maximum of 5 minutes, while the breaks between trials will be self-paced by the examinator (usually between 30s to 1 minute). The order of the conditions is randomized and counterbalanced across subjects, which was implemented to make the appearance and nature of the oddball less predictable. The same stimuli will be delivered on both visits. At the beginning of the first visit, the staircase procedure will be implemented to define the stimulation temperatures. The visit including the EEG assessment will take around 1.5 hours in total, while the VAS assessment will take around 1h per participant.

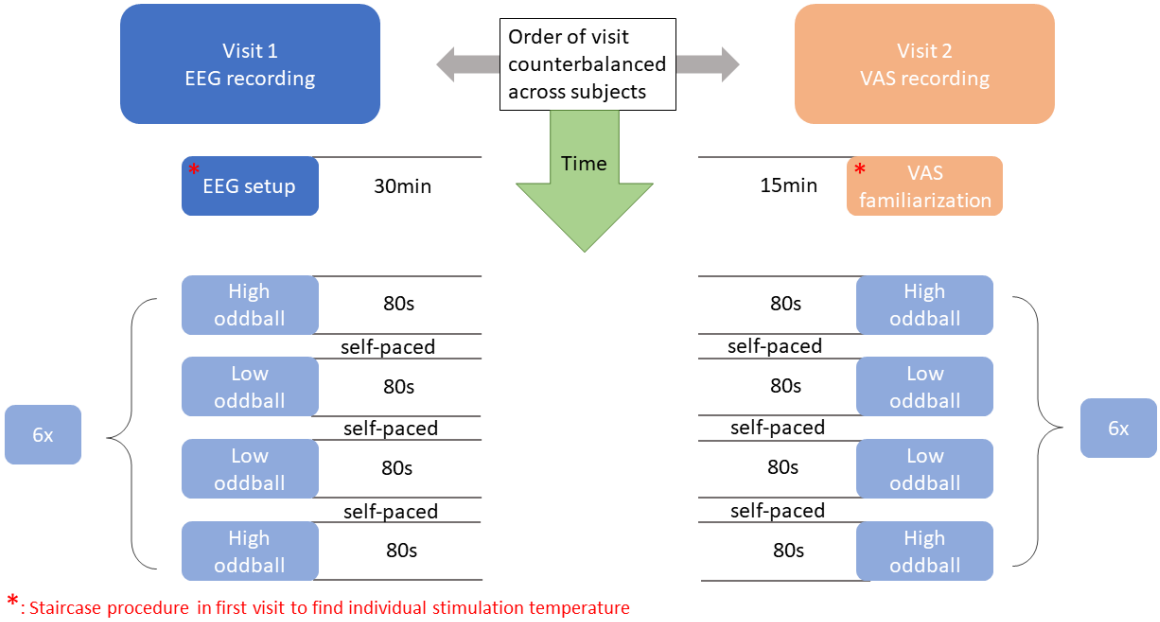


Figure 43: Illustration of the organization of the experiment. The visits will be one week apart and conducted around the same time of day and the same stimuli will be delivered in both visits. A staircase procedure will be implemented at the beginning of the first visit to find the ideal stimulation temperature, at which the experiment is tolerable but still painful throughout the entire stimulation.

2.6. EEG recordings

An elastic electrode-cap with 64 active, pre-amplified Ag-AgCl electrodes (BioSemi, Netherlands) arranged in accordance with the international 10-10 system will be used to record EEG. To maintain a clear signal, the direct-current offset will be limited to 30 mV. All electrodes will be re-referenced offline to the average electrode activity. The BioSemi ActiView software will store the recorded signal for subsequent offline analyses. Two additional electrodes will be added to the setup to record eye movements and muscular artifacts originating from the face. These electrodes will be placed between the eyebrow and on one of the zygomatic processes.

2.7. EEG analysis

The EEG recordings will be analyzed using the Letswave7 (www.letswave.org) toolbox in MATLAB (2022a The MathWorks).

2.7.1. Analysis of the phase-locked response

We will employ a frequency-tagging analysis approach (Regan, 1989) to analyze the periodic response induced by the slow sustained periodic stimuli, which will allow us to differentiate between oscillatory activity related to our stimuli and other unrelated ongoing activity (Colon, Nozaradan, et al., 2012). The frequency-tagging method is based on the notion that a periodic stimulus elicits a periodic activity which can be identified as periodic responses at the frequency of stimulation in the recorded EEG signals (Colon, Legrain, et al., 2012 ; Mouraux, Diukova, et al., 2011) (illustrated in Figure 5). This approach has been frequently used in our lab, leading to a standardized analysis approach (Colon et al., 2014 ; Colon et al., 2017 ; Mulders et al., 2020). The obtained EEG signal is first filtered using a Butterworth band-pass filter between 0.05 and ~~40~~ 30 Hz. Then, the signal will be segmented into epochs of the length of stimulation (80s), relative to the onset of the stimuli. To remove potential muscular artifacts (i.e., from eye movements), an Independent Component Analysis (Fast ICA algorithm) (Hyvarinen & Oja, 2000) will be applied, and any trial containing amplitudes larger than ± 500 µV will be removed. The remaining signal will be re-referenced to the average of the electrode set, and the waveforms will then be averaged across participants. To analyze the signal in the frequency domain, a discrete Fourier transform (FFT) (Frigo & Johnson, 1998) will be used. Finally, we will subtract at each electrode and at each frequency bin the average amplitude of the signal measured at the maximum amount of ~~2-5~~ neighboring frequencies (depending on the location of the electrode, this number varies from 2-5) to remove residual noise (Mouraux, Iannetti, et al., 2011). The peak at the frequency of the oddball stimulation (FoS) in each condition will be selected for the continuation of the analysis ($FoS_{base~~line~~}$=0.5 Hz). In a similar (visual) oddball paradigm, it has been shown that responses related to the periodic oddball are the strongest at the first 3 harmonics (i.e., $FOS_{oddball}$=0.~~0~~125 Hz, FoS_{H2}=0.~~0~~25 Hz and FoS_{H3}=0.~~0~~375 Hz) (Rossion et al., 2015). The signal with the largest response within the first three harmonics will be selected for each participant and used in the continuation of the

analysis. We will refrain from summing up any harmonics to avoid the aggregation of overlapping data between the two stimuli (Rossion et al., 2015).

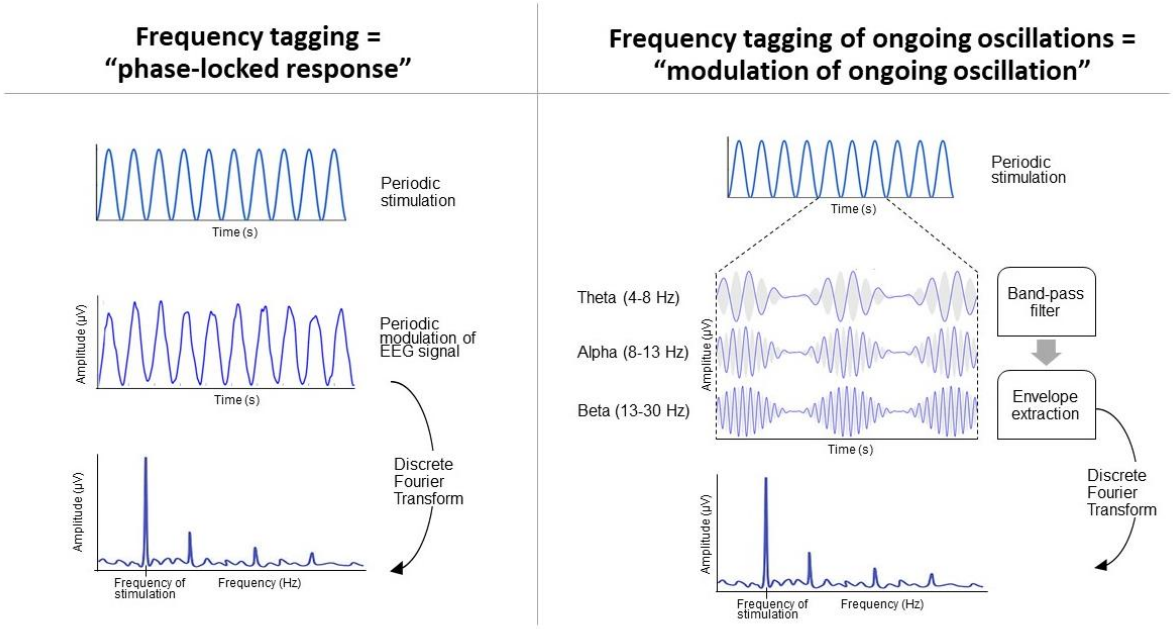


Figure 5: Illustration of the frequency-tagging method as well as its extension for the frequency-tagging of ongoing oscillations.

2.7.2. Analysis of the modulation of ongoing oscillations

The analysis of the modulation of ongoing oscillations only differs from the previously described steps in two points. To isolate the activity related to specific frequency bands (theta: 4-8 Hz, alpha: 8-~~12~~ 13 Hz, beta: 12-~~40~~ 30 Hz, in accordance with the COBIDAS recommendations (Pernet et al., 2020)), a 4th order Butterworth bandpass filter will be used to filter the EEG signal after the re-referencing step. Additionally, a Hilbert transform will be applied to estimate the envelope of the signal. The remaining steps will be the same as described in the analysis of the phase-locked response (averaging, FFT, removal of residual noise). The resulting amplitude at the $FoS_{oddball}$ and $FoS_{base~~line~~}$ in each frequency band, condition and for all electrodes will be considered for the statistical analysis. As for the phase-locked response, the first 3 harmonics of the oddball stimulus will be considered in each condition, and the harmonic with the largest modulation (i.e., Wilcoxon signed-rank test statistic) will be selected for each participant.

2.8. Statistical Analysis

Statistical analysis will be done using R Statistical Software (Version 4.1.0, R Core Team 2021) and MATLAB (2020b The MathWorks). The significance level will be set at $p<0.05$. A Kenward-Roger approximation generating appropriate type 1 error rates for smaller sample sizes will be used to test the significance of the results.

2.8.1. Behavioral data

To analyze the continuous ratings and find peaks related to the oddball in both conditions, a time-window of ~~2~~ 1-2.6s seconds after the oddball stimulus (i.e. peak of the stimulation) will be assessed and the highest rating within this window will be selected. The length of this time-window is based on Mulders et al. (2020), who reported that peaks in continuous rating followed on average between 1.35 and 2 seconds after the peak of the stimulus delivered at a frequency of 0.2 Hz as well as our own pilot data (see Supplementary Materials). The same procedure will be carried out to detect peaks associated with the baseline stimulation. The detected peaks will be aggregated (summed up and divided by the number of peaks) for each stimulus and condition. All means and standard deviations will be reported. To assess whether the rating related to the oddball differs from the rating of the baseline stimuli, a linear mixed model will be used, assessing the effect of the factors *stimulus* (baseline/oddball) and *condition* (high oddball / low oddball) and their interaction on the ratings of perceived stimulus intensity. *Subject* will be added as a random effect to adjust the intercept of the regression model for each participant. A significant interaction effect between stimulus and condition will be further tested using a pairwise comparison (t-test) to extract the difference between stimuli for each condition. Based on the assumption that the high oddball will be more salient than the baseline stimuli, we expect that they will be perceived as more ~~inten~~painful~~se~~ than the baseline stimuli. In the low oddball condition, we expect the oddball to be perceived as less ~~intense~~ painful than baseline stimuli, since the oddball will be delivered using a lower stimulus intensity.

To be able to compare the ratings related to the oddball in the two conditions relative to the baseline peaks (which are not necessarily of the same amplitude in the two oddball conditions),

the difference between baseline and oddball will be calculated for each condition (=Δhigh and Δlow). A paired t-test will be applied to compare the difference between oddballs across the conditions. We expect that the high oddball will be rated as more ~~intense~~ painful than the low oddball stimuli. ~~If saliency is driving the perception of the low oddball, it will be perceived similarly to the high oddball.~~

2.8.2. Phase-locked response

To control for a non-normal distribution of the data set and to account for potential type I error inflations due to multiple testing, a right-tailed multi-sensor cluster-based permutation test using a Wilcoxon signed-rank test as test statistic will be used to identify amplitudes at the FoS which are significantly different from zero. A Bonferroni corrected alpha level of 0.0125 (the standard alpha level 0.05 divided by the number of conditions) will be used to account for multiple testing (the median being compared to 0 at each of the 64 channels). The threshold for the cluster-based permutation will also be set to 0.0125, and 2000 permutations will be computed. The sensor connection threshold for the multi-sensor analysis will be set to 0.161, thus each channel has 4 neighbors on average. A periodic response is considered when the Wilcoxon signed-rank test (with the conditions specified above) identifies an amplitude as significantly different from zero. Electrodes showing a periodic response that are neighboring each other will be pooled and analyzed as a cluster (Hauck et al., 2015 ; Tiemann et al., 2015).

Based on the frequency-tagging premise, we expect to find a periodic response at both FoS_{base}, in both conditions. Previous investigations in our lab using a stimulation frequency of 0.2 Hz showed that this elicits a very consistent response (Colon et al., 2017 ; Mulders et al., 2020). If none of the electrodes show a significant increase in periodic response at the FoS_{base}, we will have to assume that we failed to induce a periodic modulation of the EEG signal, rendering the data unusable as the fundamental objective of the investigation was not achieved (positive control).

We further expect to find a periodic response at the $FoS_{oddball}$, in both conditions. A peak at the $FoS_{oddball}$ would show that the periodic oddball paradigm adapted from a visual stimulation paradigm also works as intended using much slower, painful stimuli. If a periodic response is found in the high oddball condition but not in the low oddball condition, we can assume that the intensity of the stimulus contributes more to the periodic response than saliency~~is mainly driven by the intensity of the stimulation~~ (since both stimuli should be salient, but only one of them is delivered at a high stimulation intensity)~~ the oddball in the high oddball condition is delivered at a higher intensity than low oddball)~~. A response larger than zero at the $FoS_{oddball}$ in the ~~high~~ low oddball condition would show that a stimulus with a lower intensity than baseline can also elicit an oddball response, potentially due to the saliency of the stimulus.

If the oddball response in both conditions is larger than zero, the relative amplitude of the oddball responses will be calculated for each condition (=Δhigh and Δlow) and compared using a paired t-test. The relative amplitude has been chosen to mitigate potential differences between the responses at the FoS'_{base}. Given the difference in oddball stimulation intensity, the baseline stimuli could also be perceived differently in the different conditions, potentially leading to non-identical responses between the conditions. If the periodic response is driven mainly by the intensity of the stimulus, we expect the amplitude of the high oddball to be larger than the low oddball. If saliency has ~~a larger influence~~an additional contribution to the periodic response ~~than the objective intensity~~, the oddballs will show a similar amplitude.

2.8.3. Modulation of ongoing oscillations

The analysis of the modulation of ongoing oscillations is identical to the analysis of the phase-locked response but will be done separately for each frequency band. Therefore, a right-tailed multi-sensor cluster-based permutation test using a Wilcoxon signed-rank test as test statistic will be used to identify the electrodes with an amplitude significantly larger than zero at both FoS' and in each condition. Corresponding to the analysis of the phase-locked response, for each frequency band, neighboring electrodes exhibiting a large modulation at the frequency of stimulation (i.e., a high test-statistic) will be pooled into clusters. We expect to find clusters

over contralateral central-parietal areas for the alpha and beta frequency band (Colon et al., 2017 ; Mulders et al., 2020) and more fronto-central for the theta frequency band (Colon et al., 2017 ; Mulders et al., 2020 ; Tiemann et al., 2015). As for the phase-locked response, we expect a periodic response at both FoS'_{base} and $FoS'_{oddball}$ in the different frequency bands and conditions. No response at the $FoS_{oddball}$ in the low oddball condition would show that the saliency of the stimulus does not contribute significantly to the modulation of ongoing oscillations and that stimulus intensity is the main contributing factor.

As for the phase-locked response, the difference between baseline and oddball will be calculated for each condition and frequency band (if both show a modulation at their FoS). Then, for each frequency band, a paired t-test will be employed to compare the peaks related to Δhigh and Δlow. If the intensity of the stimulus is the main factor in the modulation of ongoing oscillations, the amplitude for Δhigh will be larger than the amplitude for Δlow (not excluding that saliency might also influence this modulation). If saliency is more relevant than stimulus intensity, the amplitudes of the oddball in the normal and the control condition will be similar to each other.

2.8.4. Outliers

Only participants that complete both experimental sessions fully will be considered for the analysis. Further, we will remove outliers (identified using Cook's distance (Cook, 1977)) as well as data points that violate LMM assumptions of linearity and normality.

Violations of LMM assumptions will be identified using a Shapiro-Wilk test to assess the normal distribution of the data. To test the data set for homoscedasticity, Levene's test will be used. In case the data does not conform to normality, a log-transform will be applied, which conforms data to the assumption of normality by correcting right-skewed data into a more normal form (Bland & Altman, 1996). Any data point that still violates any of the assumptions after the transformation or disproportionately affects the dataset after fitting the LMM will be removed

from the data set and will not be replaced. This will lead to the exclusion of this participant from the analysis.

Cook's Distance [D] will be used to identify data points that over-proportionally influence the data set. This method calculates how much the fitted values of a given data set change if just one data point is removed. The influence of a data point is expressed in the "distance" D; the larger it is, the more influential the data point (Cook, 1977). Therefore, any data point exceeding a D of 1 will be removed from the data set. Cook's distance will be calculated for each datapoint within a condition, using a separate calculation for each condition and frequency band.

Removing outliers can be tricky and offers a certain analytical flexibility which should be mitigated as much as possible in a Registered Report (Leys et al., 2019). In case we will remove outliers from the data set, we will add the complete analysis with the full data set (no outliers removed) in the Supplementary Materials for comparison purposes.

Bibliography

Colon, E., Legrain, V., & Mouraux, A. (2012). Steady-state evoked potentials to study the processing of tactile and nociceptive somatosensory input in the human brain. *Neurophysiologie Clinique/Clinical Neurophysiology*, *42*(5), 315-323. https://doi.org/https://doi.org/10.1016/j.neucli.2012.05.005

Colon, E., Legrain, V., & Mouraux, A. (2014). EEG Frequency Tagging to Dissociate the Cortical Responses to Nociceptive and Nonnociceptive Stimuli. *Journal of Cognitive Neuroscience*, *26*(10), 2262-2274. https://doi.org/10.1162/jocn_a_00648

Colon, E., Liberati, G., & Mouraux, A. (2017). EEG frequency tagging using ultra-slow periodic heat stimulation of the skin reveals cortical activity specifically related to C fiber thermonociceptors. *NeuroImage*, *146*, 266-274. https://doi.org/https://doi.org/10.1016/j.neuroimage.2016.11.045

Colon, E., Nozaradan, S., Legrain, V., & Mouraux, A. (2012). Steady-state evoked potentials to tag specific components of nociceptive cortical processing. *NeuroImage*, *60*(1), 571-581. https://doi.org/https://doi.org/10.1016/j.neuroimage.2011.12.015

Cook, R. D. (1977). Detection of Influential Observation in Linear Regression. *Technometrics*, *19*(1), 15-18. https://doi.org/10.1080/00401706.1977.10489493

Creac'H, C., Bertholon, A., Convers, P., Garcia-Larrea, L., & Peyron, R. (2015). Effects of aging on laser evoked potentials. *Muscle & Nerve*, *51*(5), 736-742. https://doi.org/https://doi.org/10.1002/mus.24458

De Keyser, R., Mouraux, A., Quek, G. L., Torta, D. M., & Legrain, V. (2018). Fast periodic visual stimulation to study tool-selective processing in the human brain. *Experimental Brain Research*, *236*(10), 2751-2763. https://doi.org/10.1007/s00221-018-5331-2

Eccleston, C., & Crombez, G. (1999). Pain demands attention: A cognitive–affective model of the interruptive function of pain. *Psychological Bulletin*, *125*(3), 356-366. https://doi.org/10.1037/0033-2909.125.3.356

Egeth, H. E., & Yantis, S. (1997). VISUAL ATTENTION: Control, Representation, and Time Course. *Annual Review of Psychology*, *48*(1), 269-297. https://doi.org/10.1146/annurev.psych.48.1.269

Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, *39*(2), 175-191. https://doi.org/10.3758/BF03193146

Frigo, M., & Johnson, S. G. (1998, 15-15 May 1998). FFTW: an adaptive software architecture for the FFT. Proceedings of the 1998 IEEE International Conference on Acoustics, Speech and Signal Processing, ICASSP '98 (Cat. No.98CH36181),

Gross, J., Schnitzler, A., Timmermann, L., & Ploner, M. (2007). Gamma Oscillations in Human Primary Somatosensory Cortex Reflect Pain Perception. *PLoS biology*, *5*(5), e133. https://doi.org/10.1371/journal.pbio.0050133

Hauck, M., Lorenz, J., Domnick, C., Gerloff, C., & Engel, A. (2015). Top-Down and Bottom-Up Modulation of Pain-Induced Oscillations [Original Research]. *Frontiers in human neuroscience*, *9*. https://doi.org/10.3389/fnhum.2015.00375

Hyvarinen, A., & Oja, E. (2000). Independent component analysis: algorithms and applications. *Neural Netw*, *13*(4-5), 411-430. https://doi.org/10.1016/s0893-6080(00)00026-5

Iannetti, G. D., Hughes, N. P., Lee, M. C., & Mouraux, A. (2008). Determinants of laser-evoked EEG responses: pain perception or stimulus saliency? *J Neurophysiol*, *100*(2), 815-828. https://doi.org/10.1152/jn.00097.2008

Legrain, V., Bruyer, R., Guérit, J.-M., & Plaghki, L. (2003). Nociceptive processing in the human brain of infrequent task-relevant and task-irrelevant noxious stimuli. A study with event-related potentials evoked by CO2 laser radiant heat stimuli. *Pain*, *103*(3), 237-248. https://doi.org/https://doi.org/10.1016/S0304-3959(02)00451-7

Legrain, V., Perchet, C., & García-Larrea, L. (2009). Involuntary Orienting of Attention to Nociceptive Events: Neural and Behavioral Signatures. *Journal of Neurophysiology*, *102*(4), 2423-2434. https://doi.org/10.1152/jn.00372.2009

Leys, C., Delacre, M., Mora, Y. L., Lakens, D., & Ley, C. (2019). How to classify, detect, and manage univariate and multivariate outliers, with emphasis on pre-registration. *International Review of Social Psychology*, *32*(1). https://doi.org/10.5334/irsp.289

Liberati, G., Algoet, M., Santos, S. F., Ribeiro-Vaz, J. G., Raftopoulos, C., & Mouraux, A. (2019). Tonic thermonociceptive stimulation selectively modulates ongoing neural oscillations in the human posterior insula: Evidence from intracerebral EEG. *NeuroImage*, *188*, 70-83. https://doi.org/https://doi.org/10.1016/j.neuroimage.2018.11.059

Lochy, A., Van Reybroeck, M., & Rossion, B. (2016). Left cortical specialization for visual letter strings predicts rudimentary knowledge of letter-sound association in preschoolers. *Proceedings of the National Academy of Sciences*, *113*(30), 8544-8549. https://doi.org/doi:10.1073/pnas.1520366113

Mouraux, A., Diukova, A., Lee, M. C., Wise, R. G., & Iannetti, G. D. (2011). A multisensory investigation of the functional significance of the "pain matrix". *NeuroImage*, *54*(3), 2237-2249. https://doi.org/https://doi.org/10.1016/j.neuroimage.2010.09.084

Mouraux, A., Guerit, J. M., & Plaghki, L. (2003). Non-phase locked electroencephalogram (EEG) responses to CO2 laser skin stimulations may reflect central interactions between A partial partial differential- and C-fibre afferent volleys. *Clin Neurophysiol*, *114*(4), 710-722. https://doi.org/10.1016/s1388-2457(03)00027-0

Mouraux, A., Iannetti, G. D., Colon, E., Nozaradan, S., Legrain, V., & Plaghki, L. (2011). Nociceptive Steady-State Evoked Potentials Elicited by Rapid Periodic Thermal Stimulation of Cutaneous Nociceptors. *The Journal of Neuroscience*, *31*(16), 6079-6087. https://doi.org/10.1523/jneurosci.3977-10.2011

Mulders, D., de Bodt, C., Lejeune, N., Courtin, A., Liberati, G., Verleysen, M., & Mouraux, A. (2020). Dynamics of the perception and EEG signals triggered by tonic warm and cool stimulation. *PLOS ONE*, *15*(4), e0231698. https://doi.org/10.1371/journal.pone.0231698

Pernet, C., Garrido, M. I., Gramfort, A., Maurits, N., Michel, C. M., Pang, E., Salmelin, R., Schoffelen, J. M., Valdes-Sosa, P. A., & Puce, A. (2020). Issues and recommendations from the OHBM COBIDAS MEEG committee for reproducible EEG and MEG research. *Nature Neuroscience*, *23*(12), 1473-1483. https://doi.org/10.1038/s41593-020-00709-0

Ploner, M., Gross, J., Timmermann, L., Pollok, B., & Schnitzler, A. (2006). Pain suppresses spontaneous brain rhythms. *Cereb Cortex*, *16*(4), 537-540. https://doi.org/10.1093/cercor/bhj001

Regan, D. (1989). Human brain electrophysiology. *Evoked potentials and evoked magnetic fields in science and medicine*.

Roa Romero, Y., Straube, T., Nitsch, A., Miltner, W. H. R., & Weiss, T. (2013). Interaction between stimulus intensity and perceptual load in the attentional control of pain. *PAIN*®, *154*(1), 135-140. https://doi.org/https://doi.org/10.1016/j.pain.2012.10.003

Rossion, B., Torfs, K., Jacques, C., & Liu-Shuang, J. (2015). Fast periodic presentation of natural images reveals a robust face-selective electrophysiological response in the human brain. *Journal of Vision*, *15*(1), 18-18. https://doi.org/10.1167/15.1.18

Schulz, E., Tiemann, L., Schuster, T., Gross, J., & Ploner, M. (2011). Neurophysiological Coding of Traits and States in the Perception of Pain. *Cerebral Cortex*, *21*(10), 2408-2414. https://doi.org/10.1093/cercor/bhr027

Tiemann, L., May, E. S., Postorino, M., Schulz, E., Nickel, M. M., Bingel, U., & Ploner, M. (2015). Differential neurophysiological correlates of bottom-up and top-down modulations of pain. *Pain*, *156*(2). https://journals.lww.com/pain/Fulltext/2015/02000/Differential_neurophysiological_correlates_of.13.aspx

Zhang, Z. G., Hu, L., Hung, Y. S., Mouraux, A., & Iannetti, G. D. (2012). Gamma-Band Oscillations in the Primary Somatosensory Cortex—A Direct and Obligatory Correlate of Subjective Pain Intensity. *The Journal of Neuroscience*, *32*(22), 7429-7438. https://doi.org/10.1523/jneurosci.5877-11.2012

Supplementary Materials

I. Hypothesis Table and Sampling Plan

Question	*Hypothesis*	*Analysis Plan*	*Interpretation given different outcomes*
1) Is the oddball perceived as different than the baseline stimulation in both conditions (i.e., high and low oddball)?	The intensity rating of the oddball stimulation will be different than the rating for the baseline stimulation in both conditions.	LMM: rating ~ stimulus * condition + (1|subject) T-test for pairwise comparison to test the direction of the interaction (condition|stimulus)	~~*Positive control:*~~ A difference in perception between oddball and baseline would indicate that the oddball paradigm is working as intended. If no difference is perceived, the oddball ~~was not~~might not have been salient enough to change perception. ~~and we will not be able to interpret the results of this experiment.~~ As the sample size is not sufficient to detect the smallest possible effect one would still be interested in (see below), a non-significant result does not necessarily indicate that there is a definitive absence of an effect and no definitive conclusions can be drawn from a non-significant result (Dienes, 2021).
2) Does the relative peak of the rating related to the high oddball differ from the rating of the low oddball?	If the oddball perception is driven by the intensity of the stimulus, the high oddball will be perceived as more intense than the low oddball.	Paired t-test of the Δ (baseline-oddball) between high and low condition.	A difference between the ratings would show that the objective intensity of the oddball is driving the subjective perception. If the oddballs had similar peaks, it ~~w~~could indicate that the perception is rather based on the saliency of the stimulus. Yet, no definitive conclusions will be drawn from a non-significant result since the sample size is not sufficient to detect the smallest possible effect one would still be interested in.
Time-locked, phase-locked response			
3a) Does the sustained periodic stimulation lead to a periodic EEG modulation at FoS_{base} in both conditions?	The slow sustained periodic stimulation paradigm will lead to a periodic modulation of the EEG signal at the FoS_{base}.	Multi-sensor cluster-based permutation Wilcoxon signed-rank test of amplitudes at the FoS_{base}.	*Positive control:* If the expected neural activity is not induced by the baseline stimulation in the stimulation paradigm (results of the one-sided Wilcoxon signed-rank test show that the amplitude at FoS_{base} is not significantly different from zero), the fundamental assumption for using the frequency-tagging approach in this study would not be met.

4a) Does the oddball stimulation lead to a periodic modulation of the EEG signal at the $FoS_{oddball}$ in the high oddball condition?	The oddball paradigm will lead to a periodic modulation of the EEG signal at the frequency at which the oddball was presented in the high oddball condition.	Multi-sensor cluster-based permutation Wilcoxon signed-rank test of amplitudes at the $FoS_{oddball}$ in the high oddball condition.	A modulation (amplitude significantly larger than zero in Wilcoxon signed-rank test) at the frequency of the high oddball would indicate that the paradigm was successful in eliciting a periodic response related to the oddball. If no peak can be detected, the paradigm did not work as intended for the phase-locked response. No definitive conclusions will be drawn from a non-significant result since the sample size is not sufficient to detect the smallest possible effect one would still be interested in.
4b) Does the oddball stimulation lead to a periodic modulation of the EEG signal at the $FoS_{oddball}$ in the low oddball condition?	The oddball paradigm will lead to a periodic modulation of the EEG signal at the frequency at which the oddball was presented in the low oddball condition.	Multi-sensor cluster-based permutation Wilcoxon signed-rank test of amplitudes at the $FoS_{oddball}$ in the low oddball condition.	*Control condition:* A modulation (amplitude significantly larger than zero in Wilcoxon signed-rank test) at the frequency of the low oddball would indicate that an oddball with a lower stimulation intensity than the baseline stimulation is able to elicit a neural response. No peak might indicate that the ~~control~~ oddball delivered at a low stimulation intensity was not ~~enough~~ intense or salient enough to induce a periodic response. No definitive conclusions will be drawn from a non-significant result since the sample size is not sufficient to detect the smallest possible effect one would still be interested in.
5) Does the high oddball lead to a larger relative response in the EEG signal at the $FoS_{oddball}$ than the low oddball in the frequency-domain?	The amplitude at the $FoS_{oddball}$ in the high oddball condition will be similar to the amplitude at the $FoS_{oddball}$ in the low oddball condition.	Paired t-test of the difference (Δbaseline-oddball) between high and low oddball condition.	A similar amplitude of the oddball in the high and low oddball condition would ~~show~~ support the notion that the oddball response is mainly driven by the saliency of the stimulus. If the oddball in the low oddball condition leads to a smaller response compared to the oddball in the high oddball condition, it could suggest that the intensity of the stimulus ~~would indicate that the intensity of the stimulus is responsible~~ is more prominently reflected in the periodic response related to the oddball than saliency. ~~for the periodic response related to the oddball.~~ No definitive conclusions will be drawn from a non-significant result since the

			sample size is not sufficient to detect the smallest possible effect one would still be interested in.
Time-locked, non-phase-locked response			
3b) Does the sustained periodic stimulation lead to a periodic EEG modulation at FoS_{base} in both conditions?	A periodic modulation of the EEG signal will be elicited in all frequency bands for ~~both~~ the FoS_{base} in both conditions. ~~and $FoS_{oddball}$~~.	Multi-sensor cluster-based permutation Wilcoxon signed-rank test of amplitudes at the FoS_{base} ~~and $FoS_{oddball}$~~†. † One test for each frequency band (theta, alpha, beta) ~~and FoS (baseline, oddball)~~	A modulation amplitude significantly larger than zero in Wilcoxon signed-rank test) at the frequency at FoS_{base} indicates that sustained periodic stimulation leads to a periodic response in the different frequency bands (Colon et al., 2017) in both conditions. No periodic response would indicate that the sustained periodic stimulation paradigm was not successful in inducing a periodic modulation.
6a) Does the oddball stimulation lead to a modulation of ongoing oscillations at the $FoS_{oddball}$ in the high oddball condition?	The oddball paradigm will lead to a modulation of ongoing oscillations at $FoS_{oddball}$ in the high oddball condition.	Multi-sensor cluster-based permutation Wilcoxon signed-rank test of amplitudes at the $FoS_{oddball}$ in the ~~normal~~ high oddball condition.	A modulation (amplitude significantly larger than zero in Wilcoxon signed-rank test) at the frequency of the oddball would indicate that the paradigm was successful in eliciting a neural response related to the oddball. No peak at $FoS_{oddball}$ would indicate that the chosen oddball parameters were not intense or salient enough to elicit a modulation of ongoing oscillations. No definitive conclusions will be drawn from a non-significant result since the sample size is not sufficient to detect the smallest possible effect one would still be interested in.
6b) Does the oddball stimulation lead to a periodic modulation of the EEG signal at the $FoS_{oddball}$ in the low oddball condition?	The oddball paradigm will lead to a modulation of ongoing oscillations at $FoS_{oddball}$ in the low oddball condition.	Multi-sensor cluster-based permutation Wilcoxon signed-rank test of amplitudes at the $FoS_{oddball}$ in the ~~control~~ low oddball condition.	Control condition: A modulation (amplitude significantly larger than zero in Wilcoxon signed-rank test) at the frequency of the oddball would indicate that an oddball with a lower stimulation intensity than the baseline stimulation is able to elicit a neural response. No peak at $FoS_{oddball}$ might indicate that the oddball in the low oddball condition was not intense or salient enough to lead to the expected response. No definitive conclusions will be drawn from a non-significant result since the sample size is not sufficient to detect the smallest possible

			effect one would still be interested in.
7) Does the high oddball lead to a larger relative response in the EEG signal at the $FoS_{oddball}$ than the low oddball in the different frequency bands?	The amplitude at the $FoS_{oddball}$ in the high oddball condition will be similar to the amplitude at the $FoS_{oddball}$ in the low oddball condition.	Paired t-test of the Δ (baseline-oddball) between high and low oddball condition. † † One test for each frequency band (theta, alpha, beta)	A similar amplitude of the oddball in the high and low oddball condition would ~~show~~ suggest that the oddball response is mainly driven by the saliency of the stimulus. If the oddball in the low oddball condition would lead to a smaller response compared to the oddball in the high oddball condition it would ~~indicate~~ suggest that the intensity of the oddball stimulus is reflected more prominently in the corresponding modulation of ongoing oscillations than saliency. ~~oddball the intensity of the stimulus is responsible for the peak related to the oddball.~~
Abbreviations. LMM: Linear mixed model; $amplitude_{FoS}$: amplitude at the frequency of stimulation $FoS_{base~~line~~}$: amplitude at frequency of baseline stimulation; $FoS_{oddball}$: amplitude at frequency of oddball stimulation~~; amplitude;~~			

Sampling plan: To reach an overall statistical power of 0.9 with an alpha level of 0.02, 30 participants would suffice according to our data stimulation (using estimated effects based on previous investigations). To account for potential dropouts (e.g., statistical outliers, incomplete data sets) and to ensure that we will still reach out targeted power, 35 participants will be enrolled. Calculations were carried out in the software G*Power (V. 3.1.9.7.) (Faul et al., 2007) (see below). This sampling size also surpasses previous investigations investigating bottom-up modulations of ongoing oscillations (n= 21, 20) (Hauck et al., 2015 ; Tiemann et al., 2015), using a frequency-tagging approach (n=8, 15) (Colon et al., 2017 ; Mulders et al., 2020) or using periodic oddball paradigms (n = 10 to 12) (De Keyser et al., 2018 ; Lochy et al., 2015 ; Rossion et al., 2015).

Rationale for deciding the sensitivity of the test for confirming or disconfirming the hypothesis: For each statistical test, the effect size was estimated and the sample size necessary to reach the desired statistical power was calculated separately. For the EEG analysis, G*Power was used to calculate the required sample sizes for the Wilcoxon signed-

rank test as well as for the paired t-test. In summary, adopting observed effect sizes from previous investigations using similar paradigms, the proposed statistical tests require 10, 30, and 27 participants respectively to reach a power of 0.9 with an alpha level of 0.02. Therefore, to satisfy the minimum requirements for each test, we will aim for a minimum sample size of 30 participants in this experiment and enroll 35 participants in the experiment.

To control for type II error rates beyond the effects found in previous studies, the "smallest effect ones does not want to miss out on" was calculated and used as the targeted effect size for each statistical test (Dienes, 2021). To find these effect sizes, the 80% confidence interval of the expected effect was calculated and the lower bound chosen for the final expected power calculation (Perugini et al., 2014). Unfortunately, our lab does not have the resources to recruit such large sample sizes (see detailed description below). This means that for the statistical tests where the sample size is not sufficient to detect the smallest effect that we would still be interested in, no final conclusions will be made on the definitive absence of an effect in case of non-significant results (see hypotheses table).

Based on previous results from our lab (Leu et al., 2023) and results from oddball investigation in the visual field (Rossion, 2014 ; Rossion et al., 2015), we expect a large effect in our sample for the detection of a peak at the frequency of the baseline stimulation. Given a normally distributed sample, an alpha level of 0.0125 (corrected for multiple comparisons) and a one-sided Wilcoxon signed-rank test against a constant and an effect size of Cohen's d=1.4, we would need to recruit 10 participants to reach the targeted statistical power. To control for the smallest possible effect we would still be interested in, the data of ~30 participants would suffice. The effect size associated with this sample size would be d=0.69 for this statistical test.

Based on other oddball paradigm investigations (Rossion et al., 2015), we expect a medium-to-large effect size for the detection of peaks related to the oddball stimuli in the two conditions (amplitudes about half the size of the baseline responses). Given a normally distributed sample, a one-sided Wilcoxon signed-rank test against a constant, an alpha level of 0.0125, power of 0.9 and an effect size of d=0.7, we would need to recruit 30 participants to reach our

target. To test for the smallest effect we would still be interested in, the recruitment of 160 participants would be necessary.

Since the comparison between the EEG amplitudes related to the painful periodic oddballs in the two conditions using a paired t-test is rather experimental, we could unfortunately not find any data from which we could approximate an effect size. Additionally, the t-test will only be carried out in case of significant result in all Wilcoxon one-sample t-tests. This also means that the eventual results of this test will have to be interpreted with caution, and eventual negative (i.e., non-significant) results do not necessarily mean that there is no effect present, since we are not sure whether we missed small effects that we would theoretically still be interested in (Dienes, 2021).

Data on the perceived level of stimulus intensity following sustained periodic heat stimuli is scarce. As an approximation, we used the effect size of the ANOVA interaction effect reported in Mulders et al. (2020), since a similar sustained periodic stimulation paradigm was used in that investigation. The interaction was calculated between the factors *temperature* (warm, cold) and *surface* (full stimulation surface, partial stimulation surface) and had an effect size n_2^p=0.222. As we do not use cold stimuli in a separate trial, but only heat stimuli, we expect that the effect size in our sample will be smaller. We estimated an intermediate effect size of n_2^p=0.08. As sample size calculations for LMMs are not feasible in G*Power, we approximated the model using the calculation for a repeated measures ANOVA, with within factors only. 1 group was compared along 4 measurements, with a correlation among repeated measures of 0.5 and a non-sphericity correction of 1. Given the effect size we estimated, a target power of 0.09 with an alpha level of 0.02, we should test 27 participants. After transforming the n_2 into an effect size expressed in Cohen's d, the conversion table proposed in Perugini et al. (2014) was used to find the sample size needed to test for the smallest effect size that would still be interesting. This led to a recommendation of over 2000 participants for this experiment.

Finally, we estimated the effect size of the paired t-test used to assess the Δ (baseline-oddball) of the ratings between the high and low condition from the same investigation by Mulders et

al. (2020). Group-level means for peak intensity ratings were provided for the comparison of hot and cool stimuli using a large and fixed probe surface, which we used to calculate the effect size (d=0.86, G*Power). Given the estimated effect, 20 participants would suffice to reach sufficient statistical power. Yet, to find the smallest effect we would still be interested in, around 160 participants would have to be recruited.

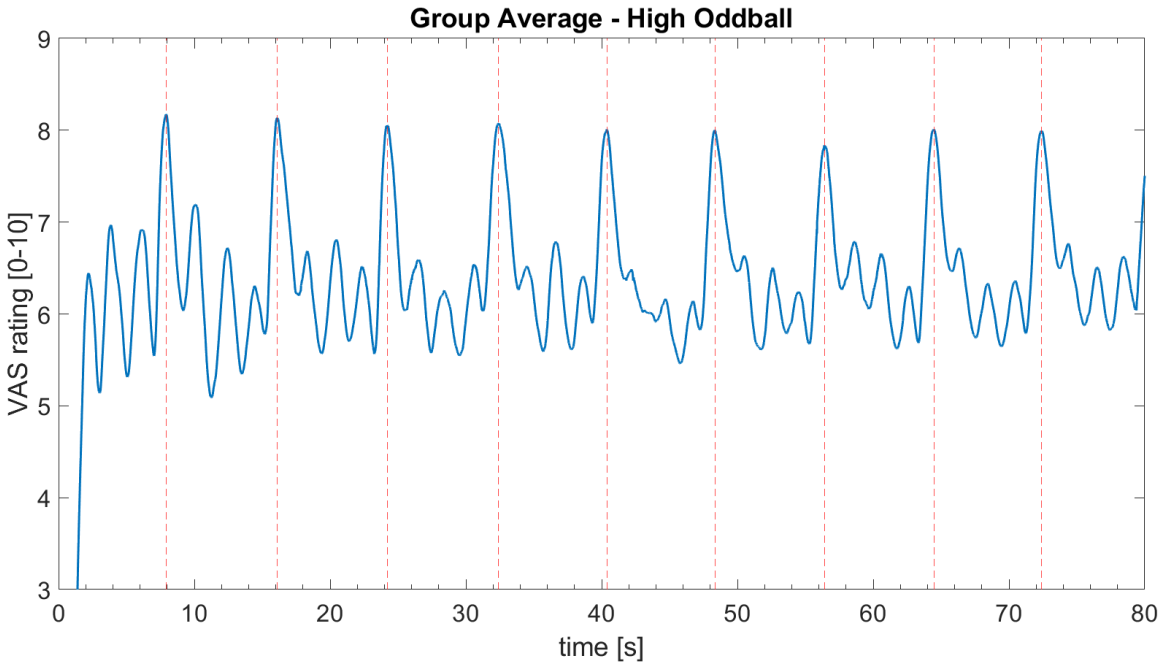
II. Pilot study

To make sure that participants will be able to trace their perception of the sustained periodic stimulation paradigm on the VAS slider (min: no perception, middle: starting to be painful, max: maximal painfulness imaginable) and perceive differences between baseline and oddball stimuli, we conducted a behavioral pilot study (10 healthy participants, 5 female, age: 24.4 ± 2.4 years old, 5 left handed) using the same parameters as described in the main manuscript. One participant had to be removed from the pilot data set due to corrupted data.

After a brief familiarization using 2 trials of warm periodic stimuli at the frequency of stimulation of the main experiment (i.e., 0.5 Hz) during which participants learned how to use the VAS slider was implemented, the staircase procedure defined the individual pain threshold was carried out.

In the main pilot experiment, 8 trials were administered in 2 blocks of 4 stimuli, counterbalanced between high and low oddball condition. The participants were asked to trace their perception of the stimulation as well as they could on the VAS. Additionally, they had to provide a verbal description of their perception of the stimuli. This was done to assess whether they would be able to perceive any sort of periodicity or oddball within each of the conditions. Across all subjects, the pain threshold was identified at 50.1°C (range: 51°C to 48.5°C). All participants were able to follow the periodic stimulation pattern with the VAS but were not able to consciously detect a pattern in the stimulation. The group average of the VAS responses following stimulation using the high oddball condition is illustrated in Supplementary Figure 1. On average, the peak of the ratings followed 1.28 ± 0.17 seconds after the peak of the oddball

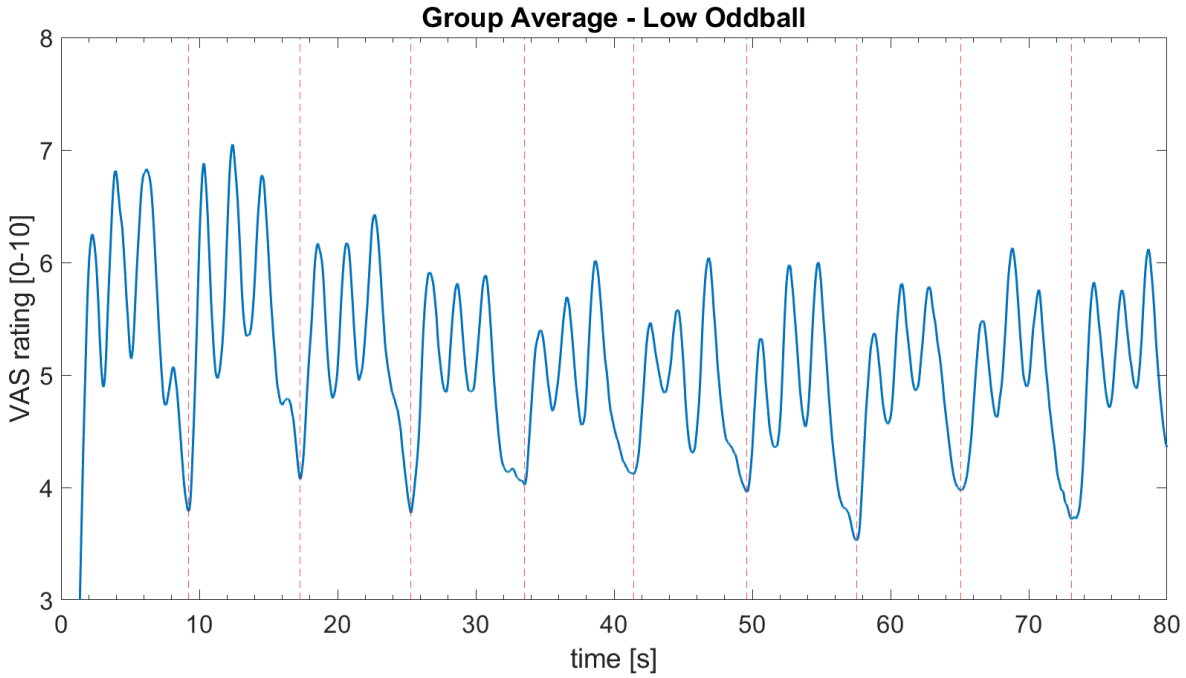
stimulation. Generally, the peaks were rated somewhat faster at the beginning of the stimulation and slowed down towards the end of the stimulation (1st peak rating: 0.92s after stimulation peak, 9th peak rating 1.39s after the peak of the stimulation). Participants clearly perceived the high oddball as more painful than baseline stimulation. As temperatures were adapted to the individual, only one person had to stop a trial due to discomfort.



***Supplementary Figure 1**:* Group and trial averages of the continuous rating provided by the participants during the stimulation on a Visual Analog Scale (VAS) during stimulation trials using a high intensity oddball stimulation. Vertical red lines represent the peak of the rating related to the oddball stimulation. The VAS scale ranged from 0 (start of perception) over 5 (start of painfulness) to 10 (maximum painfulness imaginable).

Trials delivered using a low intensity oddball were overall perceived as less painful (Supplementary Figure 2). Still, the oddball can be clearly differentiated from the baseline stimulation. The peak of the oddball ratings followed on average 2.33 ± 0.19 seconds after the peak of the oddball stimulus and didn't vary much across the duration of the stimulation. The peaks of the baseline stimulation were always perceived as painful, whereas the peaks associated with the low oddball were merely perceived as very intense (rating below 5 on the VAS).

Overall, the pilot study confirmed that both the high and low oddball stimuli can be differentiated from the baseline stimuli and lead to a periodic response in the VAS ratings. Additionally, we were able to show that even though ratings vary based on the applied stimuli, participants were not able to detect a pattern within the trials, minimizing effects of e.g., expectation. Thus, this pilot study supports that – using the proposed experimental setup –, we will be able to modulate pain perception as planned.



Supplementary Figure 2: Group and trial averages of the continuous rating provided by the participants during the stimulation on a Visual Analog Scale (VAS) during stimulation trials using a low intensity oddball stimulation. Vertical red lines represent the peak of the rating related to the oddball stimulation. The VAS scale ranged from 0 (start of perception) over 5 (start of painfulness) to 10 (maximum painfulness imaginable).

Bibliography Supplementary Materials

Colon, E., Liberati, G., & Mouraux, A. (2017). EEG frequency tagging using ultra-slow periodic heat stimulation of the skin reveals cortical activity specifically related to C fiber thermonociceptors. *NeuroImage*, *146*, 266-274. https://doi.org/https://doi.org/10.1016/j.neuroimage.2016.11.045

De Keyser, R., Mouraux, A., Quek, G. L., Torta, D. M., & Legrain, V. (2018). Fast periodic visual stimulation to study tool-selective processing in the human brain. *Experimental Brain Research*, *236*(10), 2751-2763. https://doi.org/10.1007/s00221-018-5331-2

Dienes, Z. (2021). Obtaining Evidence for No Effect. *Collabra: Psychology*, *7*(1). https://doi.org/10.1525/collabra.28202

Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, *39*(2), 175-191. https://doi.org/10.3758/BF03193146

Hauck, M., Lorenz, J., Domnick, C., Gerloff, C., & Engel, A. (2015). Top-Down and Bottom-Up Modulation of Pain-Induced Oscillations [Original Research]. *Frontiers in human neuroscience*, *9*. https://doi.org/10.3389/fnhum.2015.00375

Leu, C., Courtin, A., Cussac, C., & Liberati, G. (2023). The role of ongoing oscillation in pain perception: Absence of modulation by a concomitant arithmetic task. *Cortex*. https://doi.org/https://doi.org/10.1016/j.cortex.2023.08.005

Lochy, A., Van Belle, G., & Rossion, B. (2015). A robust index of lexical representation in the left occipito-temporal cortex as evidenced by EEG responses to fast periodic visual stimulation. *Neuropsychologia*, *66*, 18-31. https://doi.org/https://doi.org/10.1016/j.neuropsychologia.2014.11.007

Mulders, D., de Bodt, C., Lejeune, N., Courtin, A., Liberati, G., Verleysen, M., & Mouraux, A. (2020). Dynamics of the perception and EEG signals triggered by tonic warm and cool stimulation. *PLOS ONE*, *15*(4), e0231698. https://doi.org/10.1371/journal.pone.0231698

Perugini, M., Gallucci, M., & Costantini, G. (2014). Safeguard Power as a Protection Against Imprecise Power Estimates. *Perspectives on Psychological Science*, *9*(3), 319-332. https://doi.org/10.1177/1745691614528519

Rossion, B. (2014). Understanding individual face discrimination by means of fast periodic visual stimulation. *Experimental Brain Research*, *232*(6), 1599-1621. https://doi.org/10.1007/s00221-014-3934-9

Rossion, B., Torfs, K., Jacques, C., & Liu-Shuang, J. (2015). Fast periodic presentation of natural images reveals a robust face-selective electrophysiological response in the human brain. *Journal of Vision*, *15*(1), 18-18. https://doi.org/10.1167/15.1.18

Tiemann, L., May, E. S., Postorino, M., Schulz, E., Nickel, M. M., Bingel, U., & Ploner, M. (2015). Differential neurophysiological correlates of bottom-up and top-down modulations of pain. *Pain*, *156*(2). https://journals.lww.com/pain/Fulltext/2015/02000/Differential_neurophysiological_correlates_of.13.aspx